Comp. Biochem. Physiol., 1972, *Vol.* 43**B**, *pp.* 905 *to* 917. *Pergamon Press. Printed in Great Britain*

SOME STUDIES ON BIOSYNTHESIS AND FUNCTION OF TREHALOSE IN THE CRAYFISH *ORCONECTES LIMOSUS* RAFINESQUE*

GERHILD SCHWOCH†

II. Zoologisches Institut der Freien Universität, Berlin

(*Received* 11 *January* 1972)

Prom. Dat. : ~~10. FEB. 1972~~ 14. MAI 1971

Abstract—1. Biosynthesis of trehalose could be detected in the abdominal muscle, antennal gland, hind-gut, integumentary tissue and, to a lesser extent, in the stomach tissue of the crayfish *Orconectes limosus*.

2. The trehalosephosphate synthetase is not particle-bound and is inhibited by trehalose.

3. The rate of trehalose synthesis is markedly dependent upon the season.

4. The label of injected ^{14}C-trehalose and ^{14}C-glucose is distributed from the hemolymph into the organs much faster than the label of ^{14}C-maltose.

5. The increase of hemolymph trehalose after injection of eyestalk extract is accompanied by a decrease of the trehalose concentration in the abdominal muscle.

6. Trehalose in the hemolymph is rapidly converted to glucose.

7. Trehalose is used for chitin biosynthesis after conversion to glucose.

INTRODUCTION

TREHALOSE is the predominant blood sugar of insects where the metabolism of this disaccharide has been extensively studied (see Wyatt, 1967).

Our knowledge concerning the occurrence and metabolism of trehalose in crustaceans is decidedly insufficient. It has been possible to detect trehalose in the encysted embryos of *Artemia salina* (Dutrieu, 1959), in some species of isopods and amphipods (Fairbairn, 1958), as well as in different decapod crustacea (Fairbairn, 1958; Dean & Vernberg, 1965; Telford, 1968a, b; Speck, 1969). On the other hand, it has been reported that no trehalose could be detected in other decapod crustacean species (Meenakshi & Scheer, 1961; Mann, 1963; Parvathy, 1970). However, only qualitative studies of trehalose biosynthesis in crustacea (*A. salina*) have been reported (Clegg, 1965). Trehalase (E.C. 3.2.1.28, Trehalose 1-glucohydrolase) has been characterized only in the crayfish *Orconectes limosus* (Jantzen, 1967).

* This paper is from a dissertation submitted to the Fachbereich Biologie der Freien Universität, Berlin, in partial fulfillment of the requirements for the degree of Doctor of Science.

† Present address: Max-Planck-Institut für Biophysik, Abteilung Zellphysiologie, 6 Frankfurt a.M. 70, Kennedy-Allee 70, Deutschland.

In this work an attempt has been made to gain some basic knowledge of trehalose metabolism in the crayfish, especially of trehalose biosynthesis and the possible significance of trehalose for sugar transport and chitin biosynthesis.

MATERIALS AND METHODS

Animals

Crayfish (*Orconectes limosus* Rafinesque = *Cambarus affinis* Say) were obtained from local fishermen. Males (weight 15–30 g) were used exclusively in this study. The animals were kept in running tap water at 9–15°C and in a light–dark cycle of 12 hr. They were usually kept no longer than 7 days before an experiment and were fed bovine heart meat the day preceding the experiment.

Animals were denoted as being in the premolt state D_0–D_1 when they had detectable gastroliths and when setae of the new integument were already recognizable in their uropods (Drach, 1939; Stevenson *et al.*, 1968; Keller, personal communication).

Injections

Maximal volumes of 0·1 ml were injected into the pericard of the crayfish. The control, or carrier solution, was van Harreveld saline (van Harreveld, 1936), buffered with Tris–maleate to pH 7·5 (Andrews, 1967). For producing eyestalk extracts the inner tissues were removed from the cuticula, homogenized in an all-glass homogenizer with van Harreveld saline and centrifuged at 400 *g* for 5–10 min. An amount of supernatant was injected which corresponded to a half eyestalk and contained 0·1–0·2 mg protein. Radioactive solutions were injected with a micrometer-driven syringe.

Collection of blood samples

To obtain more than 0·2 ml of hemolymph one to two walking legs of the animals were cut off. Smaller amounts were taken directly by glass capillaries from the coxal joint.

Tissue preparation

In general, the animals were killed by freezing them for 20 min at −30°C. When it was necessary to halt metabolism quickly the animals were dropped into heptan at −70°C. The antennal gland was prepared without the bladder. Only that part of the integumentary tissue under the branchiostegites was used. Usually 20 per cent homogenates were prepared.

Determination of mono- and oligosaccharides

To purify the tissue homogenates protein and polysaccharide were precipitated by the addition of methanol to a final concentration of 80 per cent. The samples were kept overnight at 4°C. Lipids were removed from the supernatant with chloroform–water. The aqueous phase was deionized by the batch method with Dowex 50 (H^+) and Dowex 21 K (acetate). In some cases, in order to achieve a better radiochemical separation of trehalose, the deionized extract was also treated with 0·5 mM potassium tetraborate and Dowex 1 (tetraborate) (Khym & Zill, 1952). The purified extracts were separated by thin-layer chromatography on boric-acid-impregnated plates with glacial acetic acid–methanol–methylethylketone, 1 : 1 : 3 (v/v), as solvent system (Pastuska, 1961). The sugars were detected with the spray naphthoresorcinol–sulfuric acid (Randerath, 1965) or with orcinol–Fe(III)–chloride–sulfuric acid (Stahl, 1962) if the sprayed fraction was to be used for the counting of radioactivity.

The quantitative determination of the carbohydrates was made with anthrone (ROE, 1955); the values were corrected according to Dimler *et al.* (1952). Losses of material in the course of the purification were corrected by appropriate controls.

UNIV. LEIDEN BIBL.

Chitin determination

Homogenates of whole animals in 80% methanol were centrifuged. The pellet was treated repeatedly with hot and cold potassium hydroxide, with hydrochloric acid, with ethanol and with ethanol–dioxan, 1 : 1 (v/v). The white chitin was dried to constant weight. An aliquot was solubilized with nitric acid and its radioactivity measured as below.

Enzyme assay

The trehalosephosphate synthetase (E.C. 2.4.1.15, UDPglucose:D-glucose-6-phosphate-1-glucosyltransferase) was assayed according to Murphy & Wyatt (1965).

Tissue was homogenized with 10 mM Tris–HCl buffer, pH 7·2, and centrifuged at 20,000 *g* for 20 min at 4°C. Aliquots of the supernatant containing 0·3–0·5 mg protein were used for the assay. Standard assay conditions consisted of 50 mM Tris–HCl buffer, pH 7·0, 10 mM $MgCl_2$, 5 mM UDPG, 0·5 μCi of 20 mM glucose-^{14}C(U)-6-phosphate and enzyme in a volume of 0·125 ml. After incubation for 60 min at 25°C, the reaction was stopped by the addition of 0·5 ml methanol. Non-radioactive trehalose was added and the trehalose isolated as described and counted as below.

Incorporation rates of ^{14}C-glucose into trehalose determined by the method described were considered as an index for the activity of trehalosephosphate synthetase because it could be assumed that the trehalose-6-phosphate formed from glucose-6-phosphate and UDPG immediately was split into trehalose and inorganic phosphate as has been reported for yeast (Panek, 1962), the cellular slime mold *Dictyostelium discoideum* (Roth & Sussman, 1966) and the insects *Hyalophora cecropia* (Murphy & Wyatt, 1965) and *Phormia regina* (Friedman, 1968). This suggestion was confirmed by an experiment in which equal enzyme activities were determined using the standard assay conditions as well as after additional hydrolysis with alkaline phosphatase for 10 hr.

The indicated specific activities are based on protein determined by the method of Lowry *et al.* (1951) with bovine serum albumin as standard.

Radioactivity determination

For counting the ^{14}C-activity of isolated sugars suitable areas on the chromatogram were scraped into counting vials and mixed with 0·05–0·10 ml water and 10 ml liquid scintillator (toluene–methanol, 8 : 1·9 with 50 mg/l. POPOP and 4 g/l. PPO). Hemolymph and methanolic tissue homogenates were dissolved with 0·5 ml ethanolamine before mixing with the scintillator. For determining the ^{14}C-activity of some organs corrections for radioactivity in the hemolymph were made by double-labeling with ^{3}H-inulin. All samples were counted in the liquid scintillation counter Betamatic 40 (Tracerlab). The channel ratios or the internal standard method were used to correct for quenching.

RESULTS

Trehalose synthesis was demonstrated by isolation of labeled trehalose from the different tissues 3 hr after injection of glucose-U-^{14}C into the hemolymph. The identity of the isolated radioactive substance with trehalose was proved as follows: the area on the chromatogram coloured with naphthoresorcinol–sulfuric acid showed

(a) an identical R_f-value compared with commercially obtained trehalose,
(b) no reaction with anilic phtalic acid,
(c) after elution with distilled water and reaction according to Grassmann *et al.* (1957) no osazon formation,

(d) hydrolysis to glucose but only under extreme conditions (2 N HCl, 1·5 hr at 100°C), where

(e) this particular glucose possessed the same specific radioactivity as the thin-layer fraction denoted as trehalose.

After incubation of isolated organs with glucose-U-^{14}C it was possible to locate the hind-gut as a site of trehalose biosynthesis. Suitable conditions for the *in vitro* assay of trehalose synthesis have been ascertained using this organ.

The rate of synthesis of trehalose is linear with time up to 120 min and with protein concentration up to 0·7 mg (Fig. 1).

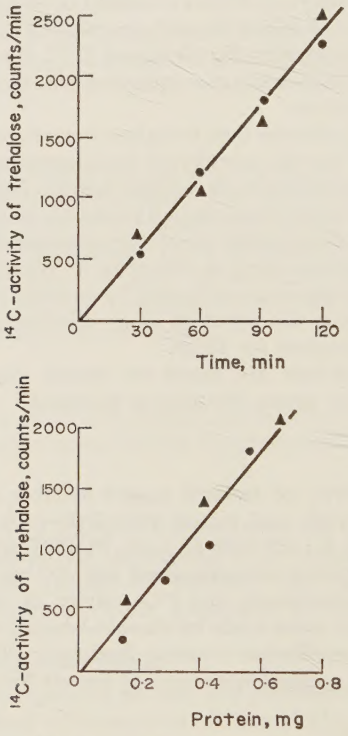

FIG. 1. Incorporation of label from glucose-^{14}C-6-P into trehalose with respect to incubation time and protein concentration. Experiments with two enzyme preparations (●, ▲) each one made from six hind-guts.

Assay of the other organs of *Orconectes* revealed synthesis of trehalose in the abdominal muscle as well as in the antennal gland, integumentary tissue and possibly in the stomach (Table 1). No synthesis of trehalose was detected in gills, gonads, heart, hemolymph, hepatopancreas, mid-gut and in the remaining part of the animal.

The trehalosephosphate synthetase does not seem to be particle-bound (Table 2). The low activity in the 78,000 *g* pellet might be due to contamination by the

TABLE 1—TREHALOSE SYNTHESIS IN THE CELL-FREE EXTRACT FROM DIFFERENT ORGANS OF *O. limosus*

	Incorporation of glucose-^{14}C into trehalose (mμmoles/min/mg)		
	Glucose-^{14}C-6-P		UDP-glucose-^{14}C
	March	May	June
Abdominal muscle	5·3	0·6	0·6
Antennal gland	2·0	N.d.	N.d.
Hind-gut		0·6	
Integumentary tissue	0·9	N.d.	<0·1
Stomach		N.d.	<0·1

For each assay, organs from six animals were pooled. N.d. = not detectable.

TABLE 2—FRACTIONATION OF TREHALOSE-SYNTHESIZING ACTIVITY IN HOMOGENATES OF ABDOMINAL MUSCLE

Fraction	^{14}C-Activity of trehalose (measured aliquot) (counts/min)	Protein in total fraction (mg)	Specific activity (mμmoles/min/mg)
Crude homogenate	1001	1·53	0·8
Supernatant,			
400 *g*, 5 min	2357	0·54	5·2
20,000 *g*, 20 min	2225	0·50	7·0
78,000 *g*, 60 min	2025	0·38	7·6
Pellet,			
20,000 *g*, 20 min	0	0·16	0
78,000 *g*, 60 min (unwashed)	213	0·41	1·1

Tissue fractions pooled from four animals were incubated under standard conditions. Values are means of duplicates.

supernatant. No washing was performed due to the instability of the enzyme preparation. The fact that the trehalosephosphate synthetase is not particle-bound is in accordance with the experiment of Murphy & Wyatt (1965) on the silkmoth *H. cecropia*.

Biosynthesis of trehalose is inhibited *in vitro* by trehalose (Fig. 2). Fifty per cent of the total enzyme activity is inhibited by 80 mM trehalose.

According to Table 1, the rate of trehalose synthesis seems to be dependent upon the season. As shown in Table 3 activity measurements on abdominal muscle

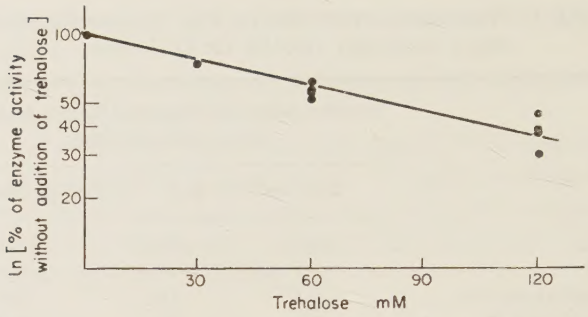

FIG. 2. Inhibition of trehalosephosphate synthetase by trehalose (abdominal muscle extracts). The value determined for the experiment with 60 mM trehalose differs from the control with $P < 0{\cdot}05$.

TABLE 3—RATE OF TREHALOSE SYNTHESIS AT DIFFERENT TIMES OF INVESTIGATION

Time of experiment	Rate of trehalose synthesis in abdominal muscle extracts (mμmoles/min/mg)		^{14}C-Activity of trehalose in the antennal gland 45 min after injection of 5 μCi ^{14}C-glucose (percentage of the per g wet weight injected activity)	
February	5·6 ± 1·4			
		$P > 0{\cdot}5$		
March	6·0 ± 0·8			
		$P < 0{\cdot}0003$		
May	0·6 ± 0·03			
		$P = 0{\cdot}07$		
Premolt (D_0–D_1)	1·4 ± 0·5		14·6 ± 3·1	
		$P = 0{\cdot}03$		$P = 0{\cdot}0008$
About 12 hr after molt	0·3 ± 0·1		3·2 ± 0·8	

Mean values ± standard deviation of four experiments.

extracts show that after a maximal rate of trehalose synthesis in February/March there is a decrease of about 90 per cent in May. In premolt animals the activity was found to be slightly increased. Immediately after molt minimal values are found. This last decrease was confirmed by measurement of the *in vivo* incorporation rate of injected ^{14}C-glucose into the trehalose of the antennal gland.

After injection of ^{14}C-labeled glucose, trehalose and maltose, the half-time of the radioactivity in the hemolymph has been determined to be 15 min for glucose, 17 min for trehalose and 220 min for maltose (Fig. 3). This behaviour of glucose and its two disaccharides is reflected in the ^{14}C-activity found in the tissues immediately after injection (Table 4).

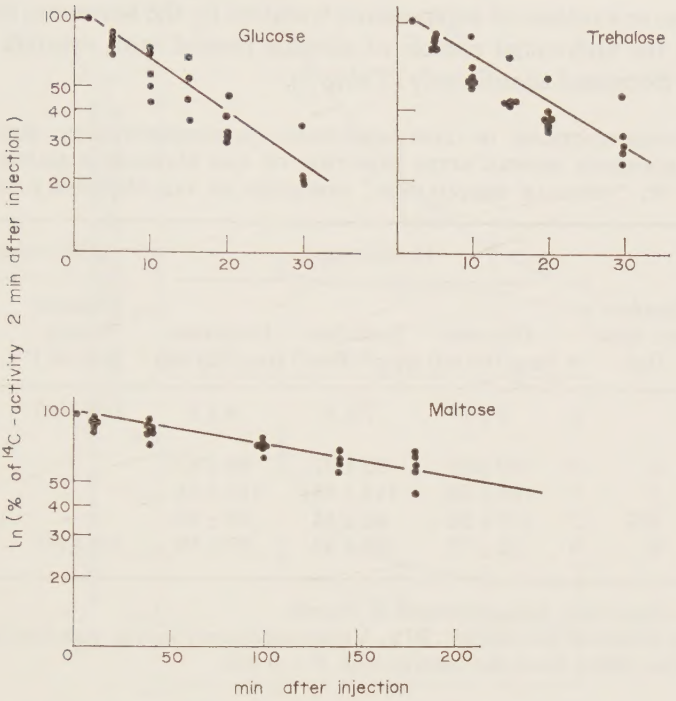

FIG. 3. Exponential decrease of ^{14}C-activity in the hemolymph after injection of glucose-U-^{14}C, trehalose-U-^{14}C and maltose-U-^{14}C.

TABLE 4—UPTAKE OF THE LABEL FROM INJECTED ^{14}C-GLUCOSE, -TREHALOSE AND -MALTOSE IN SOME ORGANS

Organ	Relative specific ^{14}C-activity (counts/min per g wet weight per g animal injected activity) after injection of		
	Glucose-U-^{14}C	Trehalose-U-^{14}C	Maltose-U-^{14}C
Abdominal muscle	0·2	0·2	<0·1
Antennal gland	1·4	1·3	0
Gills	0·7	0·7	0
Gonads	1·2	1·0	0
Hepatopancreas	1·3	1·4	<0·2
Integumentary tissue	0·9	0·7	0
Stomach/gut	0·5	0·6	0

The values are means of duplicates made 2, 4, 8 and 16 min after injection.

The level of trehalose in the hemolymph of *O. limosus* can reach remarkably high values under the influence of eyestalk hormone (Schwoch, 1969). This increase is

obviously due to a release of organ-stored trehalose by the hormone; the content of trehalose in the abdominal muscle of animals treated with eyestalk extract was found to be decreased significantly (Table 5).

TABLE 5—CONCENTRATIONS OF LOW-MOLECULAR CARBOHYDRATES IN THE HEMOLYMPH AND THE ABDOMINAL MUSCLE AFTER INJECTION OF VAN HARREVELD SALINE (CONTROL) AND 0·5 "EYESTALK EQUIVALENTS" DISSOLVED IN VAN HARREVELD SALINE

			Hemolymph			Abdominal muscle	
	Incubation time (hr)	*n*	Glucose (mg/100 ml)	Trehalose (mg/100 ml)	Galactose (mg/100 ml)	Glucose (mg/g wet wt.)	Trehalose (mg/g wet wt.)
Control		6	3 ± 1	2 ± 1	4 ± 2	0·7 ± 0·3	11·7 ± 2·8
+ Eyestalk extract	3	7	90 ± 20	95 ± 57	84 ± 47		
	5	7	108 ± 38	112 ± 38	112 ± 54		
	6·5	7	111 ± 58	86 ± 43	98 ± 65		
	8	6	82 ± 37	102 ± 43	75 ± 19	0·8 ± 0·5	5·4 ± 2·7†

The experiment was performed in March.
Mean weight of the crayfish: 20 g. Values represent mean ± standard deviation.
† Value differs from the control with $P = 0{\cdot}006$.

Suggestions that eyestalk extracts have an influence on the rate of trehalose synthesis and hydrolysis could not be confirmed by *in vivo* and *in vitro* experiments.

The trehalose released due to the action of the hormone seems to account at least partially for the hyperglucosemia which is observed after injection of eyestalk extract. This can be concluded from one experiment in which ^{14}C-glucose or ^{14}C-trehalose, respectively, was injected into the hemolymph of hormone-treated animals. Then the time course of the relative specific activities of both sugars was measured. As can be seen from Fig. 4, most of the radioactivity accumulates in the glucose fraction, even if administered as trehalose-^{14}C. Figure 4b clearly shows that by 2 hr after injection of ^{14}C-trehalose the level of labeled trehalose is lower than that of the labeled glucose. After 7 hr an equilibrium has obviously been reached. The slope of the curves indicates that a considerable part of the hemolymph trehalose has contributed to the level of hemolymph glucose. The opposite transformation occurs to a much lesser extent.

Table 6 shows that in freshly molted crayfishes ^{14}C-activity has been detected in chitin as quickly as 30 min after injection of ^{14}C-labeled glucose and ^{14}C-labeled trehalose as well as ^{14}C-labeled maltose. The lower rate of incorporation of label from injected ^{14}C-maltose compared with ^{14}C-glucose and ^{14}C-trehalose is in agreement with the longer half-time of disappearance of this label in the hemolymph (see Fig. 3). The radioactivity incorporated into chitin expressed as a percentage of the tissue radioactivity (determined by subtraction of hemolymph radioactivity from the injected radioactivity) was similar for each of the sugars.

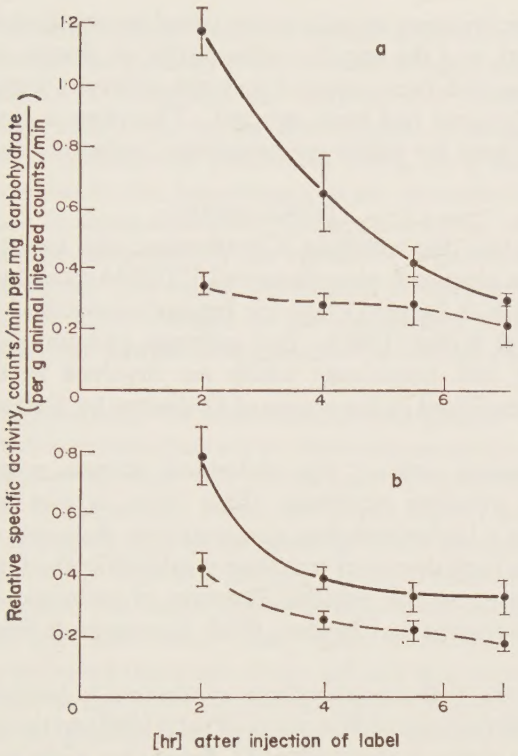
a
b
Relative specific activity (counts/min per mg carbohydrate / per g animal injected counts/min)
[hr] after injection of label

FIG. 4. Time course of ^{14}C-activity of trehalose and glucose in the hemolymph of crayfishes treated with eyestalk extract after injection of 0·3 μCi glucose-U-^{14}C (a) or trehalose-U-^{14}C (b). The animals received 0·5 "eyestalk equivalents" 1 hr before injection of the label. The experiment was performed in March. Values of (a) represent means of four experiments, those of (b) means of five experiments and vertical bars, the standard error. ——, ^{14}C-activity of glucose; – – – –, ^{14}C-activity of trehalose.

TABLE 6—INCORPORATION OF LABEL FROM INJECTED ^{14}C-GLUCOSE, -TREHALOSE AND -MALTOSE INTO CHITIN

Injected substance (1μCi)	^{14}C-Activity of chitin isolated from one crayfish (percentage of injected radioactivity)	
	30 min after injection	60 min after injection
Glucose-U-^{14}C	4·9 ± 1·6	14·4 ± 6·8
Trehalose-U-^{14}C	4·6 ± 2·1	13·9 ± 6·3
Maltose-U-^{14}C	0·5 ± 0·3	2·7 ± 0·5

The values are means ± standard deviation of values made on four animals which had molted 3 ± 0·5 hr before.

Thirty min after injection no radioactive trehalose was found in the homogenate of the entire animal, and the specific radioactivity of glucose in the animal group where ^{14}C-trehalose had been injected was not different from that of the group where radioactive glucose had been injected. Therefore it can be concluded that trehalose has been used for chitin synthesis after hydrolysis to glucose.

DISCUSSION

It now seems clear that trehalose is synthesized also in the crayfish *O. limosus* from the substrates glucose-6-phosphate and UDPG according to the scheme first postulated by Cabib & Leloir (1958) for brewer's yeast and later confirmed for insects by Candy & Kilby (1961). The enzymes phosphoglucomutase, UDPG-pyrophosphorylase and hexokinase which are involved in the supply of both substrates have been found in the organs of *O. limosus* by Keller (1964) and Jantzen (1967).

Among the tissues assayed, the abdominal muscle seems to be the most important site of trehalose synthesis. This tissue is also found to contain in autumn and spring a higher trehalose concentration than any other organ (Speck, 1969). A relatively high degree of trehalose synthesis in the hind-gut and antennal gland may be related to the possible function of trehalose as the intermediate product in glucose resorption (Sacktor, 1968; Grossman & Sacktor, 1968; Sacktor & Berger, 1969).

The fact that the trehalosephosphate synthetase is inhibited by trehalose *in vitro* agrees with the findings of Murphy & Wyatt (1965) on the silkmoth *H. cecropia*. Since in winter the abdominal muscle of *O. limosus* has trehalose concentrations up to 60–150 mMole/l. intracellular water (Jantzen, 1967) it seems possible that the *in vitro* feedback type inhibition of trehalosephosphate synthetase may be of physiological significance. This inhibition has a regulative function in *H. cecropia* because of the competition of trehalosephosphate synthetase and glycogen synthetase for UDPG. Since a relatively high glycogen synthetase activity is also found in the abdominal muscle of *O. limosus* (Jantzen, 1967) such a regulatory system seems feasible in the crayfish as well.

The seasonally dependent rate of trehalose synthesis in the abdominal muscle corresponds well with the seasonal changes in the oligosaccharide concentration in this organ as determined by Jantzen (1967).

The hyperglycemic action of crustacean eyestalk extract resembles in many ways the action of corpora cardiaca extract in insects. In the locust *Leucophaea maderae* corpora cardiaca extract stimulates the release of trehalose from the fat body as well as trehalose synthesis in this tissue (Wiens & Gilbert, 1967). In the crayfish only the former finding has been confirmed. This is possibly due to the fact that in the crayfish most of the trehalose is stored in the tissues (Speck, 1969) in contrast to insects where the major portion is localized in the hemolymph (Clegg & Evans, 1961).

The hormonal induction of trehalose release, its fast transport from the hemolymph into the organs and its rapid transformation to glucose might give

trehalose the role of an immediately available energy source for the crayfish. This could be of importance for the sudden reverse motion of the animals by contractions of the abdominal muscle. Comparably the catabolism of trehalose serves as the main energy-supplying reaction during the first seconds of insect flight (Sacktor & Wormser-Shavit, 1966; Srivastava & Rockstein, 1969). It is also more likely that sudden transient increases of the hemolymph sugar concentration which can be measured, for example, in stress situations (Kleinholz & Little, 1949; Riegel, 1960; Telford, 1968c) would be supported by the release of organ-stored trehalose than by degradation of glycogen. This would also explain why Keller (1969) was able to detect eyestalk-extract-induced changes of the enzymes of glycogen degradation and synthesis only after the glucose concentration in the hemolymph was already increased.

The fact that trehalose after its transformation to glucose is used for chitin synthesis in the crayfish is in accordance with observations on the fly *Lucilia cuprina* (Crompton & Polakis, 1969). Since our results only describe the incorporation of radioactive label from trehalose into chitin we are not able to come to conclusions about the quantitative significance of trehalose in chitin synthesis. According to Zaluska (1959), Dall (1965), Dean & Vernberg (1965), Travis (1965) and Speck & Urich (1971), the material for chitin synthesis in crustaceans should be derived from degradation of glycogen rather than from trehalose. From the results reported here and considering that most of the animals lack glucose-6-phosphatase in their muscle it would be interesting to determine if trehalose plays a specific role in the distribution of the glucose from glycogen to the locus of chitin biosynthesis.

REFERENCES

Andrews P. (1967) Über den Blutchemismus des Flusskrebses *Orconectes limosus* und seine Veränderung im Laufe des Jahres. *Z. vergl. Physiol.* **57**, 7–43.

Cabib E. & Leloir L. F. (1958) Biosynthesis of trehalose phosphate. *J. biol. Chem.* **231**, 259–275.

Candy D. J. & Kilby B. A. (1961) The biosynthesis of trehalose in the locust fat body. *Biochem. J.* **78**, 531–536.

Clegg J. S. (1965) The origin of trehalose and its significance during the formation of encysted dormant embryos of *Artemia salina*. *Comp. Biochem. Physiol.* **14**, 135–143.

Clegg J. S. & Evans D. R. (1961) The physiology of blood trehalose and its function during flight in the blowfly. *J. exp. Biol.* **38**, 771–792.

Crompton M. & Polakis S. E. (1969) The labeling of tissue components during the pharate adult life of *Lucilia cuprina*, after injection of U-^{14}C-glucose. *J. Insect Physiol.* **15**, 1323–1329.

Dall W. (1965) Studies of the physiology of a shrimp, *Metapenaeus* sp. (Crustacea: Decapoda: Penaeidae)—IV. Carbohydrate metabolism. *Aust. J. mar. Freshwat. Res.* **16**, 163–180.

Dean J. M. & Vernberg F. J. (1965) Effects of temperature acclimation on some aspects of carbohydrate metabolism in decapod crustacea. *Biol. Bull.* **129**, 87–94.

Dimler R. J., Schaefer W. C., Wise C. S. & Rist C. E. (1952) Quantitative paper chromatography of D-glucose and its oligosaccharides. *Analyt. Chem.* **24**, 1411–1414.

Drach P. (1939) Mue et cycle d'intermue chez les crustacés decapodes. *Annls Inst. oceanogr. Monaco* **19**, 103–391.

DUTRIEU J. (1959) Présence de tréhalose dans l'œuf durable d'*Artemia salina* L. *C. r. hebd. Séanc. Acad. Sci., Paris* **248**, 1038–1039.

FAIRBAIRN D. J. (1958) Trehalose and glucose in helminths and other invertebrates. *Can. J. Zool.* **36**, 787–795.

FRIEDMAN S. (1968) Trehalose regulation of glucose-6-phosphate hydrolysis in blowfly extracts. *Science* **159**, 110–111.

GRASSMANN W., HÖRMANN H. & HAFTER R. (1957) Eine quantitative Bestimmung von Kohlenhydraten als Osazone. *Hoppe-Seyler's Z. physiol. Chem.* **307**, 87.

GROSSMAN I. W. & SACKTOR B. (1968) Histochemical localization of renal trehalase: demonstration of a tubular site. *Science* **161**, 571–572.

JANTZEN H. (1967) Die Aktivitäten von Enzymen des Glykogen- und Trehalosestoffwechsels in den einzelnen Organen des Flusskrebses *Orconectes limosus* Rafinesque und Kontrollmechanismen des Glykogenstoffwechsels im Schwanzmuskel. Ph.D. thesis, Freie Universität, Berlin.

KELLER R. (1964) Die Aktivitäten von Enzymen der Glykolyse und des Citronensäurezyclus in den einzelnen Organen des Flusskrebses *Cambarus affinis* Say. Ph.D. thesis, Freie Universität, Berlin.

KELLER R. (1969) Zur hormonalen Blutzuckerregulation bei dekapoden Crustaceen. Habilitationsschrift, Freie Universität, Berlin.

KHYM J. X. & ZILL L. P. (1952) The separation of sugars by ion exchange. *J. Am. Chem. Soc.* **74**, 2090–2094.

KLEINHOLZ L. H. & LITTLE B. C. (1949) Studies in the regulation of blood sugar concentration in crustaceans—I. Normal values and experimental hyperglycemia in *Libinia emarginata*. *Biol. Bull.* **96**, 218–227.

LOWRY C. H., ROSEBROUGH N. J., FARR A. L. & RANDALL R. J. (1951) Protein measurement with the Folin phenol reagent. *J. biol. Chem.* **193**, 265–275.

MANN E. A. (1963) The nature and metabolism of the carbohydrates and lipids of *Carcinus maenas*. Ph.D. thesis, Southampton.

MEENAKSHI V. R. & SCHEER B. T. (1961) Metabolism of glucose in the crabs *Cancer magister* and *Hemigrapsus nudus*. *Comp. Biochem. Physiol.* **3**, 30–41.

MURPHY T. A. & WYATT G. R. (1965) The enzymes of glycogen and trehalose synthesis in silk moth fat body. *J. biol. Chem.* **240**, 1500–1508.

PANEK A. (1962) Synthesis of trehalose by baker's yeast (*Saccharomyces cerevisiae*). *Archs Biochem. Biophys.* **98**, 349–355.

PARVATHY K. (1970) Blood sugars in relation to chitin synthesis during cuticle formation in *Emerita asiatica*. *Marine Biol.* **5**, 108–112.

PASTUSKA G. (1961) Die Kieselgelschicht-Chromatographie von Phenolen und Phenolcarbonsäuren. *Z. anal. Chem.* **179**, 355–358.

RANDERATH K. (1965) *Dünnschichtchromatographie*. Verlag Chemie, Weinheim.

RIEGEL J. A. (1960) Blood glucose in crayfishes in relation to molt and handling. *Nature, Lond.* **186**, 727.

ROE J. H. (1955) The determination of sugar in blood and spinal fluid with anthrone reagent. *J. biol. Chem.* **212**, 335–343.

ROTH R. & SUSSMAN M. (1966) Trehalose synthesis in the cellular slime mould *Dictyostelium discoideum*. *Biochem. biophys. Acta* **122**, 225–231.

SACKTOR B. (1968) Trehalase and the transport of glucose in the mammalian kidney and intestine. *Proc. natn. Acad. Sci. U.S.A.* **60**, 1007–1014.

SACKTOR B. & BERGER S. J. (1969) Formation of trehalose from glucose in the renal cortex. *Biochem. biophys. Res. Commun.* **35**, 796–800.

SACKTOR B. & WORMSER-SHAVIT E. (1966) Regulation of metabolism in working muscle *in vivo*—I. Concentrations of some glycolytic, tricarboxylic acid cycle, and amino acid intermediates in insect flight muscle during flight. *J. biol. Chem.* **241**, 624–631.

SCHWOCH G. (1969) Einfluss von Augenstielextrakt auf Trehalose, Fruktose und Glukose in der Hämolymphe von *Orconectes limosus*. *Experientia* **25**, 1324.

SPECK U. (1969) Das Kohlenhydratspektrum in den Organen des Flusskrebses *Orconectes limosus* und seine Veränderungen im Jahresablauf. *Z. vergl. Physiol.* **65**, 51–69.

SPECK U. & URICH K. (1971) Quantitative Bedeutung der Reservestoffe für Chitinsynthese, Energiestoffwechsel und osmotische Vorgänge während der Häutung des Flusskrebses *Orconectes limosus*. *Z. vergl. Physiol.* **71**, 286–294.

SRIVASTAVA P. N. & ROCKSTEIN M. (1969) The utilization of trehalose during flight by the housefly, *Musca domestica*. *J. Insect Physiol.* **15**, 1181–1187.

STAHL E. (1962) *Dünnschichtchromatographie*. Springer-Verlag, Berlin.

STEVENSON J. R., GUCKERT R. H. & COHEN J. D. (1968) Lack of correlation of some proecdysial growth and developmental processes in the crayfish. *Biol. Bull.* **134**, 160–176.

TELFORD M. (1968a) The identification and measurement of sugars in the blood of three species of Atlantic crabs. *Biol. Bull.* **135**, 574–584.

TELFORD M. (1968b) Changes in blood sugar composition during the molt cycle of the lobster, *Homarus americanus*. *Comp. Biochem. Physiol.* **26**, 917–927.

TELFORD M. (1968c) The effects of stress on blood sugar composition of the lobster, *Homarus americanus*. *Can. J. Zool.* **46**, 819–826.

TRAVIS D. F. (1965) The deposition of skeletal structures in the crustacea—5. The histomorphological and histochemical changes associated with the development and calcification of the branchial exoskeleton in the crayfish, *Orconectes virilis* H. *Acta histochem.* **20**, 193–222.

VAN HARREVELD A. (1936) A physiological solution for freshwater crustaceans. *Proc. Soc. exp. biol. Med.* **34**, 428–432.

WIENS A. W. & GILBERT L. I. (1967) Regulation of carbohydrate mobilization and utilization in *Leucophaea maderae*. *J. Insect Physiol.* **13**, 779–794.

WYATT G. R. (1967) The biochemistry of sugars and polysaccharides in insects. In *Advances in Insect Physiology* (Edited by BEAMENT J. W. L., TREHERNE J. E. & WIGGLESWORTH V. B.), Vol. 4, pp. 287–360. Academic Press, New York.

ZALUSKA H. (1959) Glycogen and chitin metabolism during development of the silkworm (*Bombyx mori* L.). *Acta biol. exp., Lodz* **19**, 339–351.

Key Word Index—Trehalose metabolism; crustacea; *Orconectes limosus*; crustacean eyestalk hormone.